Estimation of melanin content in iris of human eye

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ABSTRACT

Based on experimental data, obtained *in vitro* from reflectance measurements and *in vivo* from digital analysis of color images of human irises, melanin content in human and bovine eye irises has been estimated. Reflectance measurements have been performed using commercially available optical multichannel spectrometer LESA-5 (BioSpec, Russia). For registration of color images digital camera Olympus C-5060 has been used. Analysis of the reflectance spectra has been performed by the method used for determination of melanin content in skin. For digital analysis of iris color images, decomposition of the images in RGB-color-coordinate system has been performed. The images have been obtained both from irises of health volunteers as from irises of patients with glaucoma. Original computer program based on Mathcad software has been developed for the analysis. The results obtained from spectral and color measurements have a good agreement each to other. In eye irises of patients with glaucoma, smaller melanin content has been obtained, and the result has been useful for development of novel and optimization of already existing methods of glaucoma diagnostics.

Keywords: iris, melanin content, glaucoma, spectroscopy, digital image analysis

1. INTRODUCTION

Knowledge of tissue optical properties is important for development of theoretical models describing the light propagation within tissues (including a human eye iris). These models can be used when designing laser therapy and diagnostic techniques, or interpretation of for the data of spectrophotometric measurements. There are numerous papers describing the methods of determination of optical properties of many types of tissues.[1-6]

Recently some diagnostic criteria of dystrophic, degenerative and inflammatory diseases of an eye iris have been based on descriptive, relative and, in many respects, subjective criteria to which the discoloration of an iris of the eye is also refered.[7-12] Now the iris eye classification is based on the data of iridochromoscopy, iridochromophotography, and biomicroscopy in polarized light.[7-13] Objective criteria of inflammatory and degenerative changes can be obtained from data of the fluorescent angiography. Unfortunately, the method is invasive and cannot be applied to all patients. Iris coloration depends mostly on the amount and depth of location of a melanin, located in the frontal mesodermal part of the iris.[9,10,12,13] The quantitative assessment of melanin content in an iris can be used as objective criterion in investigating the series of pathological conditions.[7,9,12,13] Besides, the objective data could be supplement to the existing classifications of an iris types.

In this study, we present technique for estimation of melanin content in eye iris with digital analysis of color images of the eye iris.

2. STRUCTURE AND PROPERTIES OF THE HUMAN EYE IRIS

The iris of the eye is forefront of a choroid, located in frontal in relation to a limbal plane. The iris has the form of a plate or a screen with lightly elliptical shape. The iris is attached by its root at the angle (iridocorneal) of the anterior chamber where it merges with the ciliary body and trabecular meshwork. The iris is 12 mm in horizontal diameter with a circumference of 37 mm. It is a cone shaped with the pupil margin located more anterioly than the root.[9,14,15]

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The anterior surface is divided into two zones: the ciliary zone with a breadth about 3-4 mm and pupil zone over 1-2 mm. The thickened region known as the collarette is located between these two zones. It corresponds to a plexus of the thin arteries amounting a small arterial circle.[7,9,16]

On a surface recesses (crypts, lacunas) around which vessels placed more densely are almost always seen. The anterior surface is characterized by radial streaks (straight when the pupil is contracted and wavy when dilated), and contraction furrows (more noticeable in dilated iridis).[16-18]

Iris thickness is not everywhere equal. Most of all it is expressed in the range of a small arterial circle: up to 480-550 microns, and the thinnest part - in ciliary zone - up to 350 microns.[16] Iris structure includes two mesodermal layers (superficial and deep), located in front and forming its stroma, and two epithelial pigmented layers, relating to a cerebral ectoderm and covering an iris behind. Mesodermal part includes the front epithelium, derived from flat cells, a front boundary layer, introduced by a narrow band of collagen fibers, the vascular layer formed by a connective tissue which is condensed around the radial vessels. The front boundary layer, which is called the front stromal leaf Krückmann, represents the changed iris stroma with pigment cells, that is more consistent in the structure.[9-11,17,18]

Accoding to M. Zaltsmann[9] this layer is poor for large vessels, and between pigmentary cells collagen fibrils are located with large amount of nervous terminations. The forward boundary layer thickness changes in various sites of an iris and, probably, exceeds the crypts depth. According to electronic microscopy, two basic cellular elements are located in this layer such as fibroblasts and melanocytes and a network of collagen fibrils. This layer also contains a capillary vessels network. Thus, pigment-containing melanocytes alongside with fibroblasts lied as one layer. In the same layer there are melanocyte congestions, towering above a forward boundary layer surface forming the pigmentary spots, so-called nevoses.[9-11,17]

Melanin synthesis occurs in special organoids - melanosomes, then a transformation into enzymatic-inert pigmentary granules takes place. On the chemical compound the pigment is a combination of sulfur-containing pheomelanin and sulfur-not-containing eumelanin, and the content of the pheomelanin produced by melanocytes at early and mature cells age is dominating (up to 99%). At cells ageing there is a eumelanin contents dominating.[19-24]

Melanin is put aside with granules in which bounded to protein (melanoproteides). The melanin precursor is the amino asid thyrosinum, which oxidation to dioxiphenylalanin (DOPA), and then to DOPA – quinone is catalyzed with enzyme tyrosinase. The further metamorphosises proceed without fermentative participation and result in formation of melanin which chemical structure is not established (gross formula $C_{77}H_{98}O_{33}N_{14}S$).[25-28]

The peak absorption of human melanin pigment occurs around 335 nm, and the absorption is almost completely reduced for wavelengths longer than 700 nm. The reflectance of the iris is quite constant over the range from 700 to 900 nm, although the iris looks much brighter in video images acquired in the longer wavelengths simply because the sclera and skin are much darker (less reflective) in those wavelengths. In Fig. 1 the extinction coefficient spectra[29] of pheomelanin and eumelanin are presented.

The melanocyte in submicroscopic level looks like the small oval body with prominent nucleus, nucleolus and numerous branch with a length up to 100 microns.[16-18] The branch connect cells to neighboring melanocytes, fibroblasts. The special arrangement around blood vessels is typical for melanocytes. From all vascular system of an organism only the iris has a ring pigmentary coating. Against a melanocyte of a front boundary layer – the stromal melanocyte has more expressed cytoplasm with mitochondria, both smooth and granular endoplasmatic reticular and free ribosomes. The ciliary element penetrating into a stroma of an iris usually arises from a cytoplasm. Melanocytes pigmentary granules are introduced with considerably melanosomes, being carriers of mature melanin. The granules of melanin which are located in a cytoplasm in electronic microscopic level can be traced in different stages of their development: premelanosomes, melanosomes and completely saturated with melanin granular elements which are observed in melanocytes.[9-11]

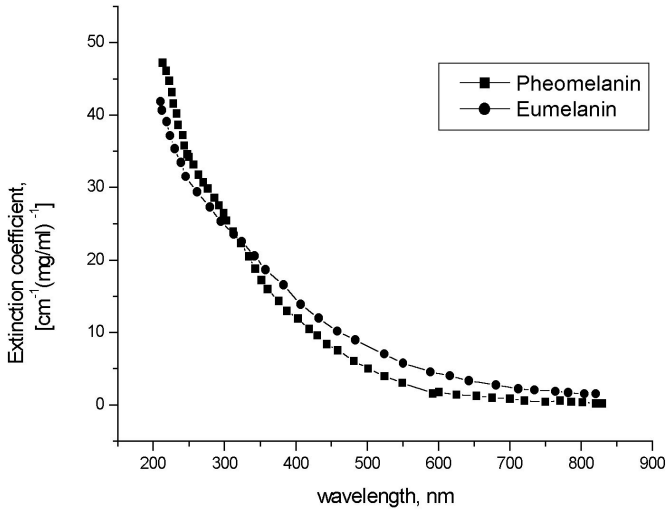


Fig. 1: Extinction coefficient spectra of melanins[29]

As to lumpy cells they are located basically in a pupillary belt of an iris, especially in a circle of a pupil sphincter. These cells have the spherical form, the cytoplasm is filled in with the melaninous granules masking a cell nucleus. The lumpy cell size is around 100 microns in the diameter. The processes length varies from 1 up to 2 microns, their width is about 0.1 microns.[16-18] Melaninous granules in a cytoplasm of a lumpy cell in submicroscopy look like the bubbles filled with very small-size melanin particles. The indicated bubbles can be considered as the secondary lysosomes which manifest pigment destruction. Lumpy cells can be seen basically in irises of older persons, for young man their detection is difficult. Melaninous granules in the residual bodies laying in stroma's back part are identical in size and form the pigment granules of the pigmentary epithelium covering an iris back surface. It should be noted, that melanin's granules size encased in the residual bodies located in front of iris stroma are equal to melanin granules size in melanocytes.[9-11]

Thus, the iris color is determined by structure of a front boundary layer, to be exact, by the concentration of pigment cells and their types. For thinner boundary layer the light-blue irises are typical saturated with pigment cells in small concentration with weak pigmentation level. Brown irises differ in thicker boundary layer and more hard pigmented cells.

3. MATERIALS AND METHODS

Estimation of melanin content in human and animal irises using experimental data obtained *in vitro* from reflectance measurements and *in vivo* from digital analysis of color images of irises has been carried out.

In this work the method of quantitative estimation of the human iris melanin content by a registration of reflectance spectra was used. As a material the irises of 2 cadaver human eyes and 10 bovine eyes have been used. Time interval from post mortem to enucleating does not exceed 24 hours. After the enucleating all samples were kept in a normal saline solution (0.9% NaCl) where they were stored up for spectroscopic measurements. All measurements were performed at room temperature about 20°C.

Eyeballs were prepared as follows: on limbs a cornea was cut off, then an iris was adjoined from choroids of an eye. The bovine irises have dark brown or black color. Color of the human irises was more diverse: from light blue up to dark brown. Irises thickness and breadth were not measured, as they have constant parameters.

Reflectance measurements have been performed in the spectral range 450-1000 nm using commercially available optical multichannel spectrometer LESA-5 (BioSpec, Russia) with the fiber-optic probe. The diameter of the

lighting and receiving fibers was 200 microns, at distance between centers of the fibers - 290 microns, mean optical penetration depth was about 150 microns, a numerical aperture of the fibers 0.22. Object illumination was performed through the optical fiber attached to the light source - halogen lamp.

The scheme of the experimental setup is presented in fig. 2. The distance between the surface of the sample (the bovine iris) and the optical fiber was 3 mm. For the measurements the iris samples were fixed in the special quartz holder having an excavation with a diameter of 12.8 mm that corresponds to the human iris diameter. Spectra were recorded by shifting of the holder with an iris sample on a meridian 9-3 hours with a walk of 1 mm. As a spectral reference $BaSO_4$ plate was used.

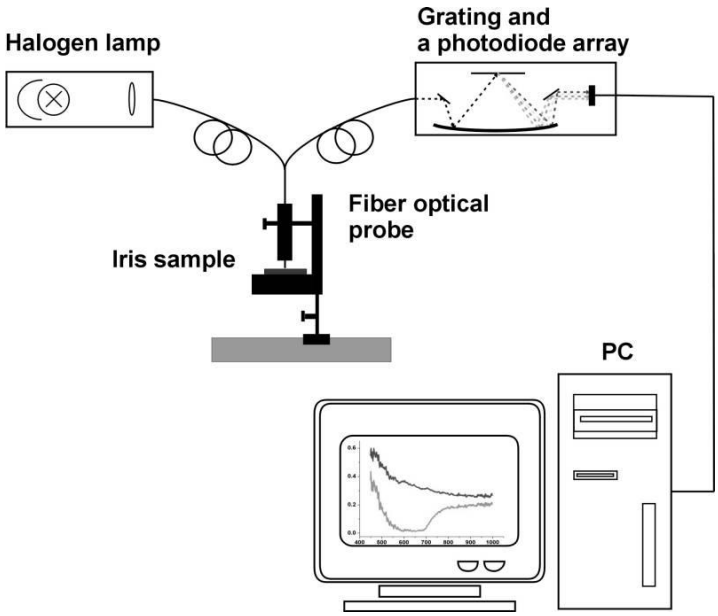
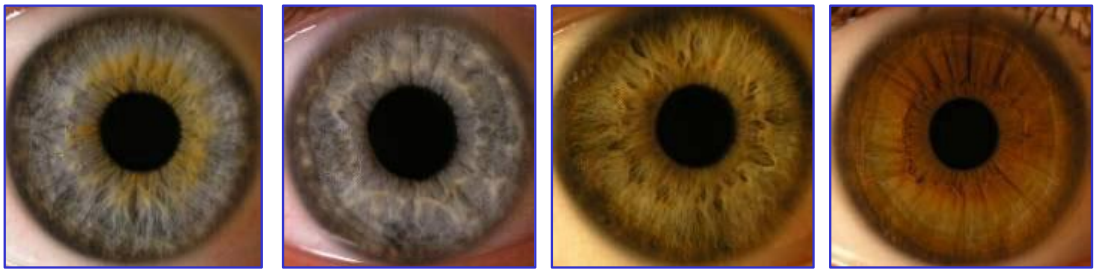


Fig. 2 Experimental setup

For registration of color images the digital camera Olympus C-5060 (Japan) was used. Its parameters: 5,100,000 effective pixels, Olympus lens from 5.7 mm to 22.9 mm. During shooting the maximum rating aperture value equals f3.3, focal length 11.5 was used. The distance between eye and camera objective was 3 cm.

For investigation the special tool stage on which fast facial fixer - for a chin and a forehead has been made, was used. It included a mobile part which supports a chin and moves up - downwards with manual screw rotation. Such equipment needed to be enforced by two lighting elements. Lighting elements were fixed on a movable basis that allowed adjusting the intensity of illumination during the measuring process. Digital camera was attached to the vertical holder fixed to movable basis that could be described as a movable device with ability to move in all directions according to tool stage. Camera can be shifted with fixed steps. It allowed us to carry out shooting separately each eye in super macro mode with manual focusing. Shooting was done at constant light exposure which was registered by metering-tool. In fig. 3 digital images of healthy human iris and human eyes with primary open angle glaucoma are presented.

a)



b)

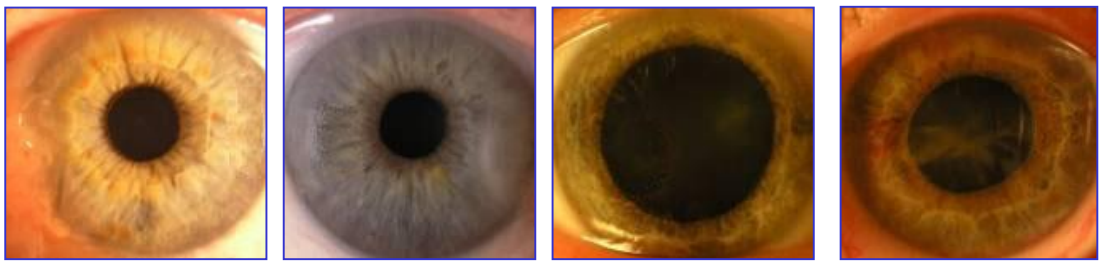
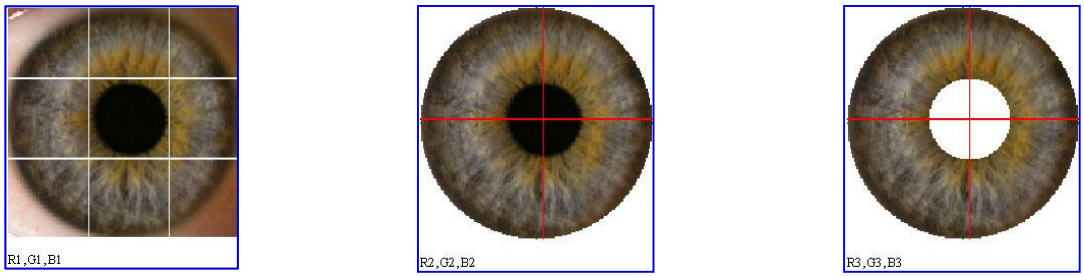


Fig. 3 Digital *in vivo* images of (a) healthy and (b) primary open angle glaucoma human eyes

To process the images of the iris we have developed the special computer program using Mathcad software (MathSoft Inc., USA). The base image was separated in three color matrixes of red, green, and blue components. As a result the averaged scans of the iris image for separated color components (red, green, and blue) corresponding to three spectral ranges for reflectance measurements have been obtained. The brightness of images of the studied irises and test-objects are expressed as the brightness from 0 to 256.

With the special markers the square form area is cut out from the received image (containing an iris of the eye) in which the circle is entered. Then nearby sclera sites are cut, the markers determine the center pupil areas which are deleted. In fig. 4 shows the main steps of image processing. The image decomposed into R, G, B color coordinates in each pixel. As a reference a white-test object has been used. For each pixel measured R, G, B values have been normalized to RGB values of the test object. The obtained reflectance values have been averaged for the whole investigated area. See fig. 4 step 3.



Step 1 Step 2 Step 3

Fig. 4 The main steps of image processing

4. RESULTS AND DISCUSSION

Quantitative estimation of chromophores content in a scattering medium is based on the analysis of reflectance and/or transmittance spectra in the selected spectral range. Absorbance of a turbid medium $A(\lambda)$ is defined as $A(\lambda) = -\ln(R(\lambda))$ or $A(\lambda) = -\ln(T(\lambda))$, where $R(\lambda)$ is the reflectance at the wavelength λ, and $T(\lambda)$ is the transmittance at the wavelength λ. These relations allow one to measure the relative changes of absorption

properties of the scattering medium. Spectrum $A(\lambda)$ for melanin can be linearly approximated in the spectral range from 620 to 1000 nm, and melanin content in tissue is determined as magnitude proportional to the linear slope of spectral dependence $A(\lambda)$ in this spectral range. Such dependence can be used for an evaluation of melanin concentration in an iris by analogy with the evaluation of the melanin content in human skin.

The slopes of such linear approximations were used for estimation of melanin concentration in the human iris in terms of DOPA melanin. Using the absorbance measurements for DOPA melanin solutions of different concentrations the following linear approximation in the range 620-720 nm was obtained[4]

$$A = -\ln(T) = C_1 - C_2 \cdot 10^{-3} \cdot \lambda, \tag{1}$$

where A is the absorbance of DOPA melanin solution; T is the transmittance of DOPA melanin solution, λ is the wavelength in nm;

$$\begin{aligned} C_1 &= 0.0688 + 0.103 \cdot C, \\ C_2 &= 0.0794 + 0.124 \cdot C, \end{aligned} \tag{2}$$

C is the DOPA melanin concentration in mg/ml. From the system of equations (2) we can obtain

$$C_1 = 0.84 \cdot C_2 \text{ and } A(\lambda) = C_2(0.84 - \lambda \cdot 10^{-3}) = C_1(1 - \frac{\lambda \cdot 10^{-3}}{0.84}). \tag{3}$$

From (3) we can obtain:

$$\begin{aligned} C_1 &= \frac{A(\lambda)}{1 - \dfrac{\lambda \cdot 10^{-3}}{0.84}}, \\ C_2 &= \frac{A(\lambda)}{0.84 - \lambda \cdot 10^{-3}}. \end{aligned} \tag{4}$$

From the system of equations (2) we can obtain

$$C = \frac{C_1 - 0.0688}{0.103} = \frac{C_2 - 0.0794}{0.124}. \tag{5}$$

Proceeding from the given technique based on measurement of reflectance, it is possible to estimate the melanin content in an iris of an eye.

In fig. 5 the reflectance spectra and in fig. 6 the optical density of brown human iris measured *in vitro* are presented.

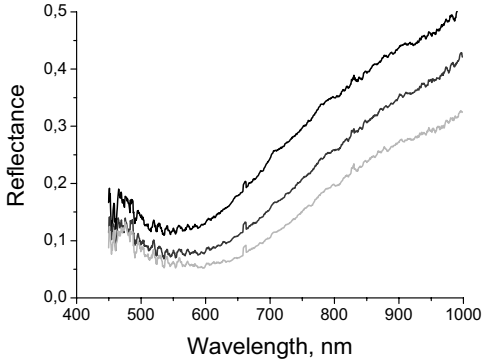


Fig. 5 The reflectance spectra (R(λ)) of brown human iris measured *in vitro* in different points of the sample

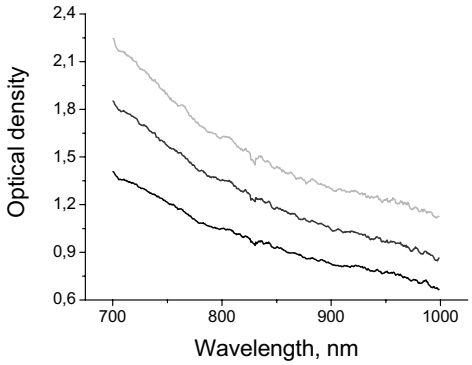


Fig.6 The optical density *D* of brown human iris calculated from measured *in vitro* reflectance spectra in different points of the sample (fig. 5); (D(λ)=–lg(R(λ))

In table 1 the mean melanin concentration values obtained from the spectral dependence optical density of bovine irises measured *in vitro* are presented.

Table 1. Mean melanin concentration values obtained from the measured *in vitro* spectral dependencies of optical density of bovine irises

Sample	C_1	C_2	Average melanin concentration in an iris, mg/ml
1	3,196	3,844	30,36
2	3,280	3,945	31,18
3	3,345	4,023	31,81
4	6,815	8,201	65,50
5	5,879	7,074	56,41
6	6,514	7,839	62,57
7	1,724	2,073	16,07
8	1,981	2,381	18,56
9	6,364	7,658	61,12
10	2,275	2,736	21,42

Basing on the results the average melanin concentration in the bovine iris has been estimated as 39.5 ± 6.2 mg/cm^3.

At examination of human iris samples, the melanin concentration has been estimated as 44.9 ± 2.1 and 46.7 ± 2.2 mg/cm^3, respectively. Thus, the mean melanin concentration obtained from the human irises has been estimated as 45.8 ± 1.2 mg/cm^3.

In tables 2 and 3 the results of digital image analysis using designed computer program are respectively presented for healthy volunteers and for volunteers with primary open angle glaucoma.

Table 2. Results of digital image analysis of healthy volunteers

Volunteer (iris color)	R_{RGB}	G_{RGB}	B_{RGB}	H_{HLS}	L_{HLS}	S_{HLS}	H_{HSV}	S_{HSV}	V_{HSV}	Melanin concentration in an iris, mg/ml
0 (blue)	136 (40.8)	112.6 (31)	84.3 (22.3)	33.8 (4.3)	110.1 (31.5)	75.1 (17.4)	33.8 (4.3)	98.4 (5.1)	136 (40.8)	21.9 (10.1)
1 (brown)	126.6 (39.3)	62.6 (18.8)	15.5 (2.7)	25.2 (1)	71.0 (20.8)	210.4 (10.4)	25.2 (1)	226.1 (6)	126.6 (39.3)	24.7 (10.9)

2 (blue)	121.4 (14.9)	84.4 (8.7)	44.6 (2.9)	30.8 (0.4)	83 (8.9)	128.4 (5)	30.8 (0.4)	166.1 (4.7)	121.3 (14.9)	24.7 (4.3)
3 (green)	106.1 (26.5)	74.2 (20)	30.9 (17.9)	34.5 (2.9)	68.5 (22)	159 (29.3)	34.5 (2.9)	189.5 (23.7)	106.2 (26.6)	30 (8.5)
4 (blue)	114 (5.1)	102.3 (3.8)	90.3 (4.1)	30.6 (4.3)	102.2 (3.8)	35.9 (7.8)	30.6 (4.3)	58 (11)	114 (5.1)	26.7 (1.5)
5 (blue)	99.8 (5.6)	81.3 (6)	67.2 (7.8)	28.5 (2.6)	83.5 (5.2)	58.4 (17.3)	28.5 (2.6)	89.5 (20.9)	99.8 (5.6)	31.3 (1.9)
6 (green)	88.7 (3.7)	76.8 (4.3)	65.8 (8.8)	28.7 (5.5)	77.3 (6.2)	47.2 (15.7)	28.7 (5.5)	74 (19.7)	88.7 (3.7)	35.2 (1.4)
7 (brown)	71 (4.9)	43.9 (1.7)	19.4 (1.4)	30.7 (0.5)	45.2 (1.8)	152.9 (5.2)	30.7 (0.5)	184.1 (5.3)	71 (4.9)	42.8 (2.4)
8 (brown)	177.7 (7.1)	101.1 (3.4)	26.1 (7.8)	29.5 (1.1)	101.9 (1.5)	204.2 (16.4)	29.5 (1.1)	220.6 (11.3)	177.7 (7.1)	11.6 (1.3)
9 (blue)	112.9 (34.1)	85.4 (25.3)	53.5 (17.8)	32.8 (2.2)	83.2 (25.7)	102.3 (15.7)	32.8 (2.2)	139.9 (15.8)	112.9 (34.1)	28.2 (10.4)
10 (green)	118.8 (24.6)	83.5 (15.6)	30.4 (10.9)	36.1 (2)	74.6 (17)	163.9 (21.5)	36.1 (2)	195 (15.5)	118.8 (24.6)	25.8 (6.3)
11 (brown)	116.3 (8.3)	71.3 (7.3)	12.8 (5.4)	33.6 (0.4)	64.6 (6.9)	220.2 (14.1)	33.6 (0.4)	232.6 (8.8)	116.3 (8.3)	26.1 (2.4)
12 (blue)	105.4 (17.4)	84.6 (12.9)	58.3 (10.7)	33.6 (2.9)	81.8 (14)	81.1 (6.7)	33.6 (2.9)	119.2 (6.9)	105.4 (17.4)	29.8 (6)
13 (green)	110.8 (5.7)	70.7 (3.6)	20.3 (4)	33.3 (0.9)	65.5 (3.8)	184 (13.2)	33.3 (0.9)	211.2 (9.1)	110.8 (5.7)	27.7 (1.7)
14 (brown)	77.6 (6.4)	39.7 (3.1)	8.6 (2.6)	26.9 (1.4)	43.1 (2.8)	203.1 (11.8)	26.9 (1.4)	222.4 (8.2)	77.6 (6.4)	39.8 (2.7)
15 (blue)	124.4 (17.7)	105.6 (17.5)	86.2 (18)	31.5 (2)	105.3 (17.7)	55.8 (12.1)	31.5 (2)	82.9 (17.3)	124.4 (17.7)	24 (5.4)
16 (blue)	128.9 (16.1)	111.8 (12.9)	95.8 (9.1)	30.9 (2.2)	112.3 (12.5)	44.4 (8)	30.9 (2.2)	68.1 (8.2)	128.9 (16.1)	22.7 (4.4)
17 (brown)	87 (24.4)	47.7 (10.6)	8.4 (1.4)	29.7 (1.5)	47.7 (12.9)	214,4 (18.2)	29.7 (1.5)	230.2 (9.3)	87 (24.4)	36.6 (9.6)
18 (green)	146.8 (28.1)	88.7 (18.4)	22 (9.8)	32 (0.5)	84.4 (18.8)	202.1 (16.7)	32 (0.5)	221.2 (10.9)	146.8 (28.1)	18.6 (6.6)
19 (green)	112.7 (16.2)	71.6 (5.7)	22 (3.7)	33 (2.5)	67.4 (8.6)	177.4 (15.8)	33 (2.5)	206.8 (11.6)	112.7 (16.2)	27.3 (4.9)

Table 3 Results of digital image analysis of patients with open angle glaucoma

Patient (iris color)	R_{RGB}	G_{RGB}	B_{RGB}	H_{HLS}	L_{HLS}	S_{HLS}	H_{HSV}	S_{HSV}	V_{HSV}	Melanin concentration in an iris, mg/ml
1 (brown)	129 (11.2)	59 (6.1)	6 (1.4)	25.7 (0.7)	67.5 (6.2)	243.2 (2.5)	25.7 (0.7)	246.2 (1.6)	129 (11.2)	22.6 (2.9)
2 (blue)	116.3 (7.6)	71.5 (5.9)	20.4 (9)	31.7 (3)	68.3 (4)	192.3 (32.4)	31.7 (3)	214 (21.8)	116.3 (7.6)	26.1 (2.2)
3 (blue)	122.9 (16.2)	76.3 (10.7)	14.9 (5.8)	34 (2.5)	68.9 (10)	213.1 (16.9)	34 (2.5)	228.7 (10.3)	122.9 (16.2)	24.4 (4.8)
4 (brown)	130.5 (14.5)	59.8 (8.6)	6 (2.3)	25.7 (1.1)	68.2 (8.2)	241.5 (4.4)	25.7 (1.1)	245.7 (2.9)	130.5 (14.5)	22.3 (3.9)
5 (blue)	119.6 (7.6)	74 (10.1)	26.9 (23.9)	29.9 (2)	73.3 (10.8)	178.8 (67.7)	29.9 (2)	199.6 (52.9)	119.6 (7.6)	25.1 (2.2)
6 (blue)	128.6 (10.7)	81.7 (7.3)	30.4 (12.7)	31.2 (1.2)	79.5 (8.7)	172.3 (29.9)	31.2 (1.2)	200.1 (24)	128.6 (10.7)	22.7 (2.8)
7 (blue)	136.4 (8.5)	115.4 (8.1)	98.6 (9.3)	27.9 (2.6)	117.5 (7.3)	45.8 (13.2)	27.9 (2.6)	71.8 (17.4)	136.4 (8.5)	20.7 (2.1)
8 (green)	137.2 (18.5)	83 (6.9)	24.4 (9.6)	31.2 (1.9)	80.8 (8.7)	191.4 (26.1)	31.2 (1.9)	213 (19.5)	137.2 (18.5)	20.6 (4.3)

9 (green)	181.7 (39.4)	117.7 (36.9)	48.7 (34.8)	30.9 (1.1)	115.2 (37)	193.2 (24)	30.9 (1.1)	195.4 (36.6)	181.7 (39.4)	11.5 (7.5)
10 (blue)	98.3 (15.3)	81.5 (14.4)	66.5 (14.9)	28.4 (2.7)	82.4 (14.7)	55.9 (13.4)	28.4 (2.7)	87.9 (17.9)	98.3 (15.3)	32.1 (5.4)
11 (blue)	113.3 (14.2)	97.6 (11.8)	85.3 (13.2)	30.7 (3.4)	99.3 (13.6)	41.8 (4.7)	30.7 (3.4)	67.5 (7.3)	113.3 (14.2)	27.1 (4.2)
12 (brown)	128.8 (11.8)	58.3 (6.5)	9.5 (3)	24.3 (0.5)	69.2 (7.2)	233.3 (6.1)	24.3 (0.5)	240.3 (3.8)	128.8 (11.8)	22.7 (3.2)

In tables 4 and 5 data, averaged from tables 2 and 3 are presented. From table 4 it is seen that maximal melanin concentration has been obtained for brown eyes. For blue and green eyes the melanin content is smaller.

Table 4. Mean melanin concentration in healthy human iris measured *in vivo* (from digital analysis of color images)

Color of eyes	Melanin concentration, mg/cm3
blue eyes	26.2±3.4
brown eyes	30.3±11.7
green eyes	27.4±5.4

Table 5 Mean melanin concentration in human iris with glaucoma measured *in vivo* (from digital analysis of color images)

Color of eyes	Melanin concentration, mg/cm3
blue eyes	25.5±3.6
brown eyes	22.5±0.2
green eyes	16.1±6.4

From table 5 it is seen that maximal melanin concentration is observed for blue eyes in contrast to data of table 4. So, melanin content in human and animal irises using experimental data obtained *in vitro* from reflectance measurements and *in vivo* from digital analysis of color images of irises was estimated. In irises of eyes with glaucoma the mean content of melanin is less in comparison with one in irises of healthy eyes. The most difference was obtained for brown and green color eyes.

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